MICROBIAL EXTRACTION OF BITUMEN FROM ATHABASCA OIL SAND

BY

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INTRODUCTION

In the 1950's the Department of Mines and Technical Surveys in Canada (now Energy, Mines and Resources or EMR) undertook a variety of investigations pertaining to the recovery of bituminous values from Athabasca tar sand using a cold water, solvent-surfactant approach. In addition to a pilot plant operation (1), studies were made by Montgomery and co-workers (2) on the effects of approximately 60 wetting agents in a laboratory scale cold-water, solvent-surfactant extraction of bitumen from Athabasca tar sand. One conclusion from these studies was that surfactants with HLB (hydrophilic-lipophylic balance) values (3) of 6-8 were most useful in terms of producing a bituminous extract which was free of water and clay while also resulting in a high overall yield. The cold-water, solvent-surfactant process for tar sand extraction has several potential advantages over present hot-water extraction technology. These include a reduction in the dispersion of clay particles (with a concomitant reduction in effluent disposal problems), and a reduction in the energy required for the extraction step (although the total energy budget of an extraction plant depends on the final design of all components.

Microbes (bacteria, fungi) are known to utilize hydrocarbon substrates such as kerosene or components of crude oil as a primary source of carbon for growth and metabolism. The solubility of most hydrocarbons in water or microbial growth media is usually negligible, and it appears that many hydrocarbonoclastic microorganisms overcome this limitation to the availability of their primary foodstuff by producing extracellular materials which adsorb, emulsify or wet the hydrocarbon phase, in order to increase contact area and to produce sub-micron droplets which can be phagocytized (4). Certain microorganisms produce surface active materials in the absence of hydrocarbons, such as *Bacillus subtilis* which produces subtilysin and of course, many biological lipids are surface active.

Recently, we have been studying the production of surface active agents by hydrocarbonoclastic microbes (5,6,7,8) and the utility of these agents in the cold-water extraction of Athabasca tar sand (9). In these studies, approximately 80 microbial cultures which were isolated from a variety of natural environments and which are capable of utilizing hydrocarbons as their primary carbon source were screened for growth rate, genetic stability and surfactant production.

Crude preparations of microbial surfactant were tested for their ability to stimulate the separation of bitumen from the mineral matter in a variety of samples of Athabasca tar sand. Within the scope of the test system which has been developed, microbial surfactants act as successful tar sand separation agents and are comparable in effectiveness with synthetic surfactants while holding the promise of reduced production costs through continuous production from a hydrocarbon feedstock in a single processing step.

EXPERIMENTAL

Microbiological and Chemical

Pure strains of microbes were cultivated in a mineral salts medium (pH 7.0) containing $NaNO_3$ (2%), K_2HPO_4 (1%), KH_2PO_4 (0.5%), KCl (0.1%), $MgSO_4$ (0.05%), $CaCl_2$ (0.01%), $FeSO_4$ (0.01%), ethylenediamene tetraacetic acid (0.001%), kerosene (Imperial Oil Number 9 refined oil (4%)) plus vitamins and minerals as needed by the various strains. Growth was studied either in 50 ml of the above medium in 500 ml shake flasks (rotated at 200 rpm with a 1.5" eccentricity) or in a New Brunswick 28 litre fermentor. Plate counts were used to determine viable cell number and biomass was considered to be that material which was retained by a 0.22μ pore diameter Millipore filter. Certain bacteriological identification tests were performed using the API-20 and API-50 test systems. (API Laboratory Products, 4008 Cote Vertu, St. Laurent, P.Q. H4R 1V4).

The surface tension of fermentation broth or solutions of crude surfactant was measured with a Fisher surface tensiometer (Autotensiomat) and is taken as the maximum of the stress-strain curve. Critical micelle concentrations (CMC) were measured and are expressed as follows: the surface tensions of a serial dilution series were measured and plotted as a function of the log of the concentration expressed as a percent of the original (%,V/V). The concentration at which saturation occurs is the CMC. Critical micelle concentrations expressed in this way are an inverse measure of the concentration of surfactant in the fermentation broth or crude extract and have proven to be highly useful in the optimization of surfactant production. Emulsification was tested by violently mixing equal volumes of an aqueous surfactant solution and a liquid hydrocarbon, allowing the mixture to settle for 24 hours and recording the percent of the total volume occupied by emulsion. Droplet size has not been studied at this time.

Tar Sand Extraction Test

In order to maximize the differentation between the effects of various surfactants, tar sand was tested for extraction in a low shear test system. In this test, 5.0 g tar sand was placed in a 500 ml flask with 50 ml of the aqueous surfactant solution to be tested. This mixture was shaken as described above for 48 hours and quantitatively analyzed for bitumen separation from sand. The shaking procedure resulted in the fractionation of bitumen into 4 fractions (Figure 1): 1. Surface oil fraction, which was collected by mopping the surface with a siliconized (Siliclad, Clay-Adam) glass fibre filter paper; 2. Residual tar sand balls, which were collected on a 40 mesh screen; 3. Sand + Clay fraction, which was collected on Whatman # 41 filter paper; and 4. Emulsified bitumen which was extracted from the filtrate with toluene. The bitumen content

of each fraction was determined by extraction with toluene and taking the O.D. at 700 nm and referring to a standard curve. A summary of the bitumen distributed in control (no surfactant) tests is given in Table 1 (± standard error of the mean of 10 observations).

TABLE 1

SUMMARY OF BITUMEN DISTRIBUTION FOLLOWING LOW SHEAR AQUEOUS EXTRACTION IN THE ABSENCE OF SURFACTANTS (Control values)

FRACTION	TAR SAND SAMPLE 1	TAR SAND SAMPLE 2
Original bitumen content (%w/w) percent of total	7.8 ± 0.3	10.2 ± 1.0
Bitumen in:*		
(a) surface fraction (1)	18.6 ± 0.3	0.6 ± 0.2
(b) residual fraction (2)	54.0 ± 5.0	97.7 ± 0.7
(c) sand + clay fraction (3)	27.1 ± 3.8	1.3 ± 0.6
(d) emulsified fraction (4)	0.3 ± 0.2	0.4 ± 0.1
Bitumen concentration in:		
(a) surface fraction (1)	100	100
(b) residual fraction (2)	30 ± 4.6	12 ± 1.8
(c) sand + clay fraction (3)	3 ± 0.5	1 ± 0.3

* Numbers refer to fractions of Figure 1.

RESULTS AND DISCUSSION

Surfactant Production

Table 2 gives the surface tension and CMC of whole fermentation broth for 5 selected microbial cultures which utilize kerosene for growth. Surfactant production in both shake-flask and batch fermentations reaches a sharp peak early in the growth cycle for cultures 1 and 2

TABLE 2

MICROBE	STRAIN	MINIMUM SURFACE TENSION (dynes/cm)	CMC (% OF WHOLE BROTH)
1. *Corynebacterium sp*	OSGB1	28	0.05%
2. *Pseudomonas, sp*	Aspha 1	31	6%
3. *Candida lipolytica*	GA	33	90%
4. *Vibrio, sp.*	Chry-B	65	100%
5. *Corynebacterium, sp*	CD1	32	0.5%

The most generally useful technique for recovering the surface active components has been extraction by exhaustive emulsification. In this method, whole fermentation broth is first centrifuged (10,000 Xg, 15 min.) to remove cells and is then mixed with an equal volume of kerosene or hexadecane and shaken violently. The resulting mixture separates into aqueous phase, an emulsion phase and a kerosene phase (certain cultures require dilution with distilled water). The emulsion is separated and mixed with more hydrocarbon and distilled water and the extraction is repeated; in addition, the aqueous phase is extracted with more hydrocarbon and the emulsion fractions are pooled. Following a triple extraction, the emulsion is lyopholyzed to give the crude product. Biochemical fractionation of this crude surfactant mixture is underway and indicates that there are several surface active components. Table 3 gives the CMC and surface tensions (γ), interfacial tensions (γH,kerosene) spreading tensions (γs) and Gibbs surface excess (Γ) for 5 selected crude microbial surfactants. Spreading tension is directly related to HLB, although no attempt has been made to determine the HLB by other means. Emulsification as a function of the chain length of the emulsified hycrocarbon is given in Figure 2 for one of the microbial surfactants.

TABLE 3

SURFACIAL PROPERTIES OF MICROBIAL SURFACTANTS AT THE CRITICAL MICELLE CONCENTRATION

MICROBE	CMC (%,w/v)	γ (dynes/cm)	γH (dynes/cm)	γS (dynes/cm)	Γ (pMoles/cm^2)
1	0.015	49	9	22	845
2	0.02	55	20	1	256
5	0.2	34	25	-15	673

Large scale (20 litre) production of microbial surfactant has been studied for Culture 1 and the results are given in Figure 3. The most remarkable feature of the fermentation is that surfactant concentration in the broth increases 100-fold over an approximately 5 hour period and then drops off to lower values. The meaning of this in terms of microbial growth is uncertain at this time, but it is correlated with maximum oxygen consumption, carbon dioxide formation, the formation of a yellow compound and the movement of the microorganisms from the aqueous to the hydrocarbon phase.

Crude microbial surfactants have been tested for toxicity using the *Daphnia magna* test system (10) and some of the results are given in Table 4. Microbial surfactants have in general proven to be a source of nutrition to the *Daphnia* and death rates in the absence of surfactant exceed those with surfactant, demonstrating a complete lack of toxicity at the concentrations tested (all are near the CMC). Results for several synthetic surfactants are included for comparative purposes.

TABLE 4

SURFACTANT	CONCENTRATION (ppm)	% MORTALITY ABOVE CONTROLS, 48hrs.
1	100	-10*
2	200	-4*
5	2000	65
Petrostep-A-50	900	100
Standamid	1050	100
Unamide JJ-35	100	100

* Greater survival rate than controls

Tar Sand Extraction

Table 5 gives the tar sand distribution results for 5 selected cultures. In these tests, tar sand was treated with a 0.02% solution of whole microbial broth for 48 hours. Two effects predominate: either (1) the microbial surfactant induces the release and flotation of bitumen as a surface slick or floating droplets, or (2) the microbial surfactant enhances the ablation of clean sand and clay particles from the tar sand balls, resulting in tar balls of increased bitumen concentration. The latter effect was noted and studied by Imperial Oil (11) and their process, which lacked surfactants, is termed sand reduction. The flotation of bitumen is given as the percent of total bitumen in the surface fraction and the effect of sand reduction is given as the bitumen concentration in the residual tar sand. All of these microbial surfactants compare favourably with the synthetic surfactants, especially if one considers that the synthetic surfactants are pure, while the microbial surfactants are present only as a small percentage of the total crude extract.

TABLE 5

TAR SAND SEPARATION BY 0.02% (v/v) WHOLE FERMENTATION BROTH CONTAINING MICROBIAL SURFACTANTS

MICROBE	BITUMEN DISTRIBUTION FOLLOWING LOW SHEAR TREATMENT OF TAR SAND (SAMPLE 2)				
	1	2	3	4	BITUMEN CONCENTRATION IN 4
1	0.6	96.9	2.1	0.4	17.9
2	3.7	85.9	10.1	0.3	17.0
3	3.6	95.7	0.6	0.1	34.9
4	2.7	94.5	2.5	0.3	18.4
5	1.3	97.5	0.9	0.3	25.7
Petrostep-A-50	0.0	93.0	0.0	7.0	15.7

CONCLUSIONS

Microbial surfactants provided by hydrocarbon fermentations are effective in tar sand separation by a cold-water process, and compare well with synthetic surfactants in their ability either to cause flotation of the bitumen or to cause ablation of sand and clay from the bitumen. The usefulness of these surfactants in a solvent-surfactant, cold-water extraction process is under study and

preliminary results indicate that these surfactants cause increased yield in a laboratory-scale extraction process. The surfactants have a high affinity for kerosene-water mixtures, which may result from biological selection for growth in such mixtures.

ACKNOWLEDGEMENTS

This work was sponsored by the Department of Energy, Mines and Resources of Canada. We wish to thank Dr. D. S. Montgomery for many useful discussions. Miss P. Bowerman and Mrs. S. Krause provided expert technical assistance.

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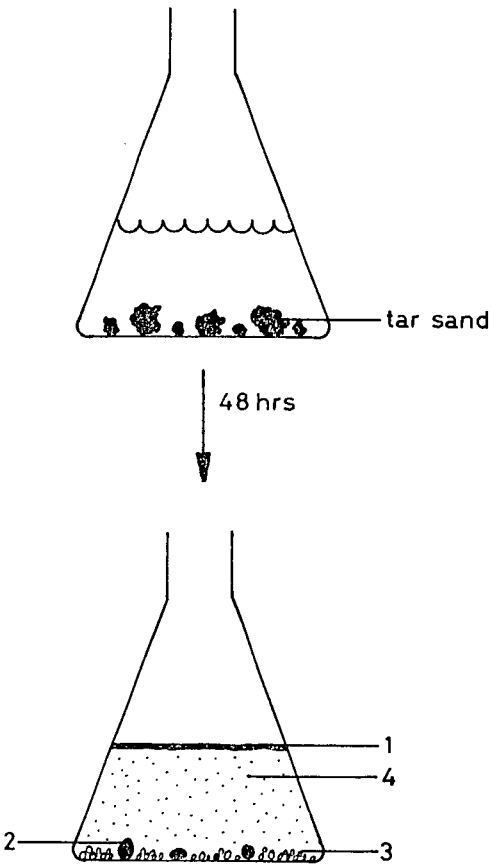

Figure 1

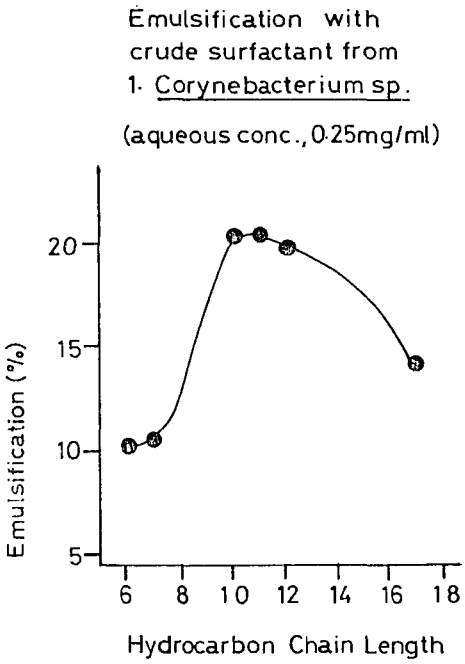
Emulsification with
crude surfactant from
1. Corynebacterium sp.
(aqueous conc., 0·25mg/ml)
Emulsification (%)
20
15
10
5
6
8
10
12
14
16
18
Hydrocarbon Chain Length

Figure 2

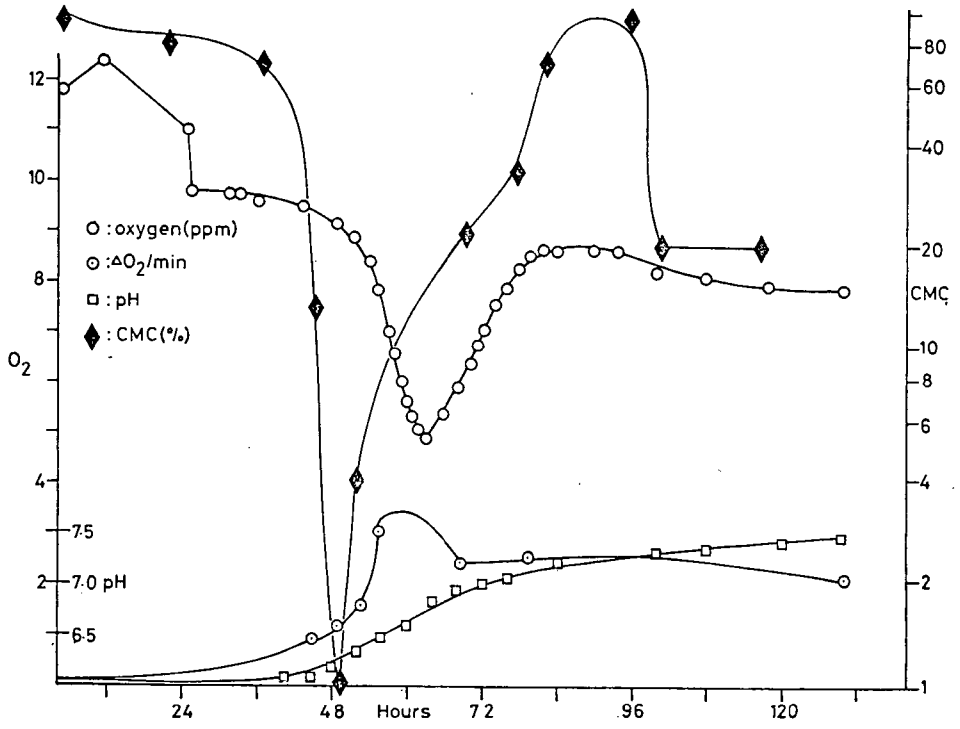
O : oxygen(ppm)
⊙ : ΔO2/min
□ : pH
♦ : CMC(%)
O2
12
10
8
4
2
7·5
7·0 pH
6·5
80
60
40
20
CMC
10
8
6
4
2
1
24
48
Hours
72
96
120

Figure 3